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Genetic and morphological evidence indicates the persistence of Japanese mitten crab mitochondrial DNA in Europe for over 20 years and its introgression into Chinese mitten crabs

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Abstract

Cryptic biological invasions are largely unrecognised, leading to an underestimation of the number of invading taxa and their potential impacts. The Chinese mitten crab, *Eriocheir sinensis*, is a highly invasive species with serious economic and ecological impacts in Europe. Recently, mitochondrial DNA (mtDNA) of the Japanese mitten crab, *E. japonica*, has been discovered in populations from The Netherlands, Poland and Germany, but the taxonomic status and time of introduction of specimens carrying this mtDNA are uncertain. To this end, we investigated the morphology and variation of the mitochondrial cytochrome c oxidase subunit I (COI) gene of mitten crabs collected in central-western Europe between 1998 and 2020. Mitten crabs from Belgium harboured a Japanese mitten crab COI haplotype in 33% to 65% of individuals, even in our earliest samples from 1998. All other studied populations carried only Chinese mitten crab COI haplotypes. Morphologically, many of the juvenile Belgian mitten crabs showed intermediate traits

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between the two species, while all investigated adult mitten crabs, regardless of their mitochondrial haplotype or country of origin, were morphologically assigned to *E. sinensis*. This intermediate morphology of the juveniles and genetic-morphological discrepancy of adults suggests that Japanese mitten crabs introgressed with Chinese mitten crabs, which could have happened both before and after the introduction of mitten crabs to Europe. A specific Chinese mitten crab COI haplotype, found in Belgium, was previously only known from Vladivostok (Russia), where Chinese and Japanese mitten crab hybrids naturally occur. This Far East region is, therefore, a plausible source for at least part of the mitten crab mitochondrial diversity in Belgium.

Keywords
Belgium, introgression, mitochondrial DNA, mitten crab, morphometrics, museum collections

Introduction

Global expansion in travel and trade has greatly increased the number of invasive species (Everett 2000). However, both the numbers of invaders and the impacts of these species may be underestimated as a result of cryptic invasions (Carlton 1994). Cryptic invasions and potential admixture between species may be occurring in one of the "world's 100 worst invasive species", the Chinese mitten crab, *Eriocheir sinensis* H. Milne Edwards, 1853 (Lowe et al. 2000). Over 100 years ago, it arrived in Europe via ballast water of large shipping vessels and has become an unprecedented nuisance with staggering ecological and economic effects (Peters and Panning 1933; Clark et al. 1998; Rudnick et al. 2000; Herborg et al. 2003, 2005; Dittel and Epifanio 2009). Mitten crabs (*Eriocheir* spp.) are catadromous species, reproducing in seawater, but spending most of their life cycle in freshwater (Peters and Panning1933; Clark et al. 2006; Naser et al. 2012; Schoelynck et al. 2021). The Chinese mitten crab is indigenous to East Asia, where it is distributed along the coast of the East China Sea, Yellow Sea and Bohai Gulf up to Vladivostok in Russia (Wang et al. 2008; Sui et al. 2009; Xu et al. 2009; Kang et al. 2018).

In a global assessment of mitten crab diversity, Hayer et al. (2019) synthesised all available sequences of the mitochondrial gene for cytochrome oxidase c subunit I (COI hereafter) for mitten crabs from GenBank and the Barcoding of Life Data System (BOLD, https://www.boldsystems.org/). They found that some specimens of presumed Chinese mitten crabs collected in The Netherlands, Poland and Germany between 2009 and 2015 yielded COI sequences of the Japanese mitten crab, *E. japonica* (De Haan, 1835). The Japanese mitten crab is indigenous to the main Japanese islands, eastern and southern parts of the Korean peninsula and Vladivostok in Russia (Xu et al. 2009) and had previously not been known to establish non-native populations. We set out to investigate the extent of *E. japonica* genetic diversity throughout Europe since 1998 and to evaluate the potential evidence for hybridisation between Chinese and Japanese mitten crabs in Europe using morphological comparisons.

Methods

A total of 141 mitten crabs were collected for morphological and genetic assessment by local fishermen or hand-caught between 1998 and 2020: 65 crabs from the Rivers Eider, Elbe, Weser and the Kiel Canal in northern Germany, 20 crabs from the Vistula Lagoon in Poland and 56 crabs from Oostende (North Sea) and the Schelde River Basin in Belgium (Table 1). They were frozen or immediately preserved in 75% to 99% ethanol. Specimens collected in Belgium between 1998 and 2005 are deposited in the National Taiwan Ocean University (NTOU). Tissue vouchers of all remaining specimens are preserved in the Zoological Museum of Kiel University, where also a subset of whole specimens from Germany, Belgium and Poland, collected between 2019 and 2020, is stored.

We extracted genomic DNA from about 1 mm^3 of pereopod muscle tissue with the Chelex method (Walsh et al. 1991) or from ~ 25 mg of pereopod muscle using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Initially, we amplified COI with the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). However, the PCR product was successfully sequenced only consistently with the LCO primer despite clear amplicons on the agarose gel. An alignment of publicly available mitochondrial genomes of *Eriocheir* showed mutations in the HCO primer region that could have influenced sequencing success. Consequently, a new reverse primer Eri-HCO2198 (5'-TAA ACT TCT GGG TGA CCG AAA AAT CA-3') was designed for amplification and sequencing to improve sequencing success. Samples collected

Table 1. Sampling information.

Country	Sampling location	GPS coordinates	Sampling dates	No. individuals	Sampling site ID	Catalogue no.
Germany	Geesthacht (Elbe River)	53.4261°N, 10.3710°E	23.10.2009	9	EL09	Cr 3207, Cr 3209
			2019–2020	10	EL20	Cr 3687
	Horst (Eider River)	54.3160°N, 9.1901°E	30.11.2009	2	EI09	Cr 3222
			30.4.2020	5	EI20	Cr 3688
	Kiel (Kiel Canal, Kiel Fjord)	54.328751°N–54.3727°N, 9.9641°E–10.1496°E	2008–2009	8	KF09	Cr 3224, Cr 3227, Cr 3201, Cr 3216
		54.3410°N–54.3699°N, 9.7349°E–10.1389°E	2019–2020	21	KF20	Cr 3689, Cr 3690
	Bünzau (Aukrug)	54.0890°N, 9.7970°E	31.07.2008	1	AU08	Cr 3215
	Thedinghausen (Weser River)	52.9828°N–52.9819°N, 9.0272°E–9.0436°E	2019	9	WE19	Cr 3514, Cr 3515, Cr 3516, Cr 3517, Cr 3518
Poland	Vistula Lagoon	54.4657°N, 19.7574°E	10.2020	20	PO20	NA
Belgium	Oostende (North Sea)	51.2281°N, 2.9509°E	08.1998	6	BE98	NTOU B00134
			08.2005	10	BE05	NTOU B00133
	Grobbendonk (Kleine Nete River, Greater Schelde River Basin)	51.1802°N, 4.7390°E	2018–2020	34	BE20	NA
	Doel (Schelde River)	51.3162°N, 4.2676°E	02.09.2020	3	BE20	NA
	Bergenmeersen (Schelde River)	51.02211°N, 3.9645°E	20.10.2020	3	BE20	NA

NA: not available.

in 1998 and 2005 were amplified following the protocol of Xu et al. (2009). For the samples collected between 2008 and 2020, we decreased the PCR mixture volume and lowered the PCR annealing temperature. Each 25 µl amplification reaction consisted of 2 µl DNA template, 1x Roti-Pol TaqS Mix (Carl Roth, Karlsruhe, Germany), 0.5 µM of each primer (Biomers, Germany) and 10 µg bovine serum albumin (Carl Roth). The PCR was carried out at 94 °C for 5 min, then 35 cycles of 95 °C for 30 sec, 47 °C for 60 sec and 72 °C for 60 sec and a final extension at 72 °C for 10 min. The amplified product was sequenced on an Applied Biosystems 3730xl DNA Analyzer in the Institute of Clinical Molecular Biology, Kiel University, Germany (IKMB). We trimmed, aligned and error-checked sequences in Geneious software v. 9.8.1 (Kearse et al. 2012). In addition to the newly-generated COI sequences, we also included the COI data of Hänfling et al. (2002) and the data accumulated by Hayer et al. (2019) for northern, western and central Europe. Otto (2012) reported six additional haplotypes unique to northern Germany that were subsequently included in the study of Hayer et al. (2019) as H12 to H17. We recovered none of these haplotypes, neither in the samples we collected between 2018 and 2020 from the same localities nor in the museum samples that were collected by Thurid Otto herself between 2008 and 2009. These haplotypes are possibly erroneous and all of Otto's sequences were, therefore, excluded from subsequent analysis. Haplotypes were called using the function 'haplotype' of the 'haplotypes' package (Aktas 2015) in the R environment (R Core Team 2019) and a haplotype network was constructed with the function 'parsimnet' of the same package. Haplotypes were labelled following Hayer et al. (2019). We tested for changing proportions of *E. japonica* haplotypes over time using a Pearson's chi-squared test for equality of proportions (function 'prop.test') in the R environment.

For the morphological assessment, we evaluated four presumably species-specific characteristics of the carapace in juvenile and adult mitten crabs that were genotyped at the COI locus: the ratio of carapace width to length, the shape of the infraorbital region, the epi-and protogastric crest and the markedness of the fourth lateral tooth, which were scored as either *E. sinensis*-like or *E. japonica*-like (Guo et al. 1997; Sakai 2013) (Table 2, Fig. 1). We considered crabs adult when they had reached a carapace width of 40 mm, following Peters and Panning (1933). We tested if differences in the character state of the infraorbital region, the crest and the extent of the fourth lateral spine were related to individual size measured as carapace width, sampling locality (Germany vs. Belgium) or COI haplotype (*E. japonica* vs. *E. sinensis*) with a logistic regression. The model was implemented with the function 'glm', specifying the error family as 'binomial' in the R environment.

We conducted a morphometric analysis, based on 27 landmarks (Fig. 1A) on the carapaces of a subset of the genotyped individuals using the 'geomorph' package v. 4.0.1 (Baken et al. 2021) in the R environment. Specifically, we first took pictures of each specimen's carapace using a digital camera, digitised landmarks with the function 'digitize2D', conducted a Generalised Procrustes analysis with the function 'gpagen' and a Principal Component Analysis (PCA) 'gm.prcomp' on the Procrustes-transformed landmark coordinates. We tested for significant ef-

Table 2. Selected species-specific carapace characteristics distinguishing *Eriocheir sinensis* and *Eriocheir japonica* according to Guo et al. (1997) and Sakai (2013). Characteristics are highlighted in Fig. 1.

Trait	*Eriocheir sinensis*	*Eriocheir japonica*
Infraorbital region	With four distinct teeth	Granulated, 4-lobed, separated medially by shallow sinus
4th lateral tooth of carapace	Present	Small or rudimentary, sometimes reduced to a granule
Carapace width : carapace length ratio	1.08 (relatively smaller)	1.12 (relatively larger)
Epi- and protogastric crest	Very strong, high and sharp	Low, weak, blunt

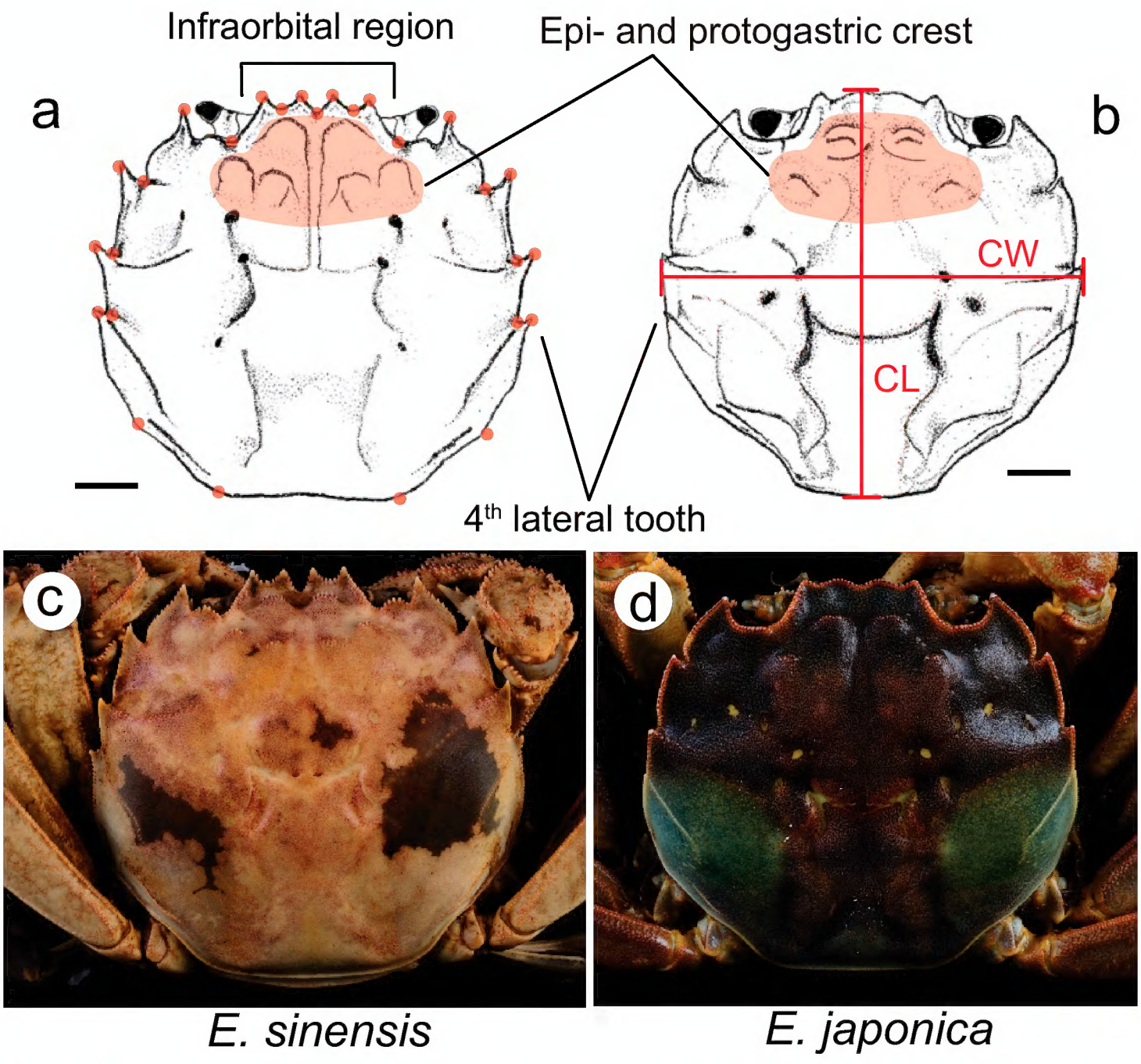

Figure 1. Carapace morphology of the mitten crab species *Eriocheir sinensis* (**A, C**) and *E. japonica* (**B, D**). Line drawings (**A, B**) are reproduced from Sakai (2013) **A** the red dots are the landmarks used in the morphometric analysis **B** the lines indicate where we measured carapace length (CL) and carapace width (CW). The two photographed specimens were collected in China **C** and Japan **D** and deposited at NTOU under voucher numbers B00132 **C** and B00135 **D**.

fects of country of origin, carapace width and their interaction with a Procrustes analysis of variance with 1000 permutations using the function 'procD.lm' of the geomorph package.

Results

A total of 141 specimens were newly sequenced at the COI locus in our study (Table 1). Together with 76 previously-published sequences, the final alignment contained 217 sequences of 511 bp length, from which we identified six haplotypes, five of which had previously been reported from the introduced range (Fig. 2). In addition to the previously reported *E. sinensis* haplotypes H1, H2, H3, H4 and the Japanese mitten crab haplotype H1, two Belgian specimens from 2020 carried the haplotype H18 (Fig. 2), which had only been previously reported from Vladivostok, Russia by Xu et al. (2009).

In total, 38 specimens carried a single *E. japonica* haplotype (Fig. 2), specifically the *E. japonica* haplotype H1 as defined by Hayer et al. (2019). To avoid confusion with the *E. sinensis* haplotype H1, which is also present in Europe, we omit the haplotype designation for *E. japonica* from here on. The mitochondrial DNA (mtDNA) of *E. japonica* was already present in our earliest samples from Belgium collected in 1998, with a frequency of 33% (two out of six specimens, Fig. 3A) and in 50% of the Belgian samples from 2005 (five out of ten specimens). Between 2018 and 2020, 65% of the investigated Belgian crabs had the *E. japonic*a haplotype (26 out of 40 specimens, Fig. 3C). Despite the apparent increase, the proportion of individuals with a *E. japonica* haplotype did not differ significantly between years (Chi-squared = 2.56,

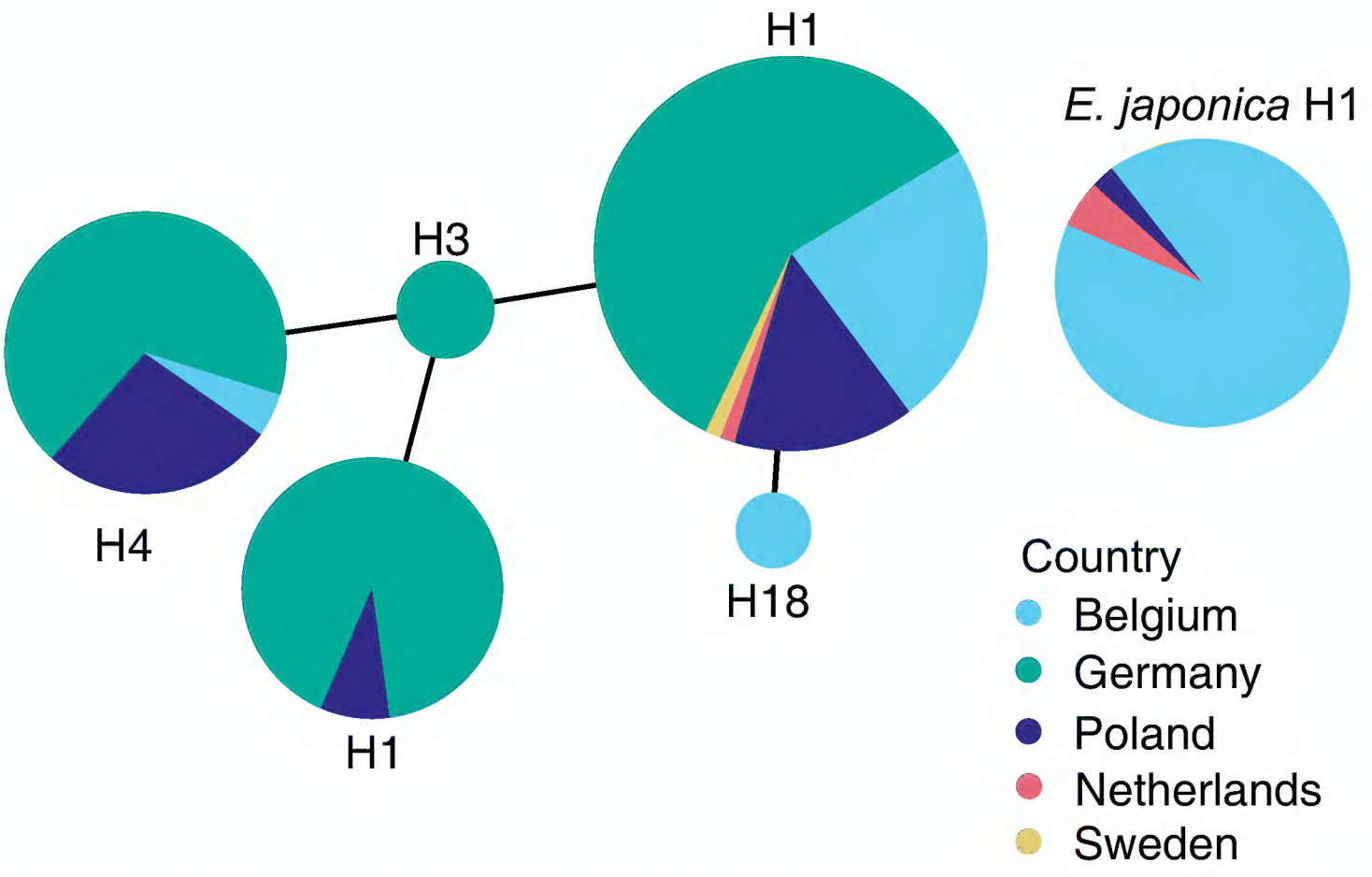

Figure 2. Haplotype network of all analysed sequences from the introduced European range. Each circle represents one haplotype. Size of the circles is proportional to the number of individuals carrying the respective haplotype. Colours denote the sampling locality. The haplotypes were named following Hayer et al. (2019).

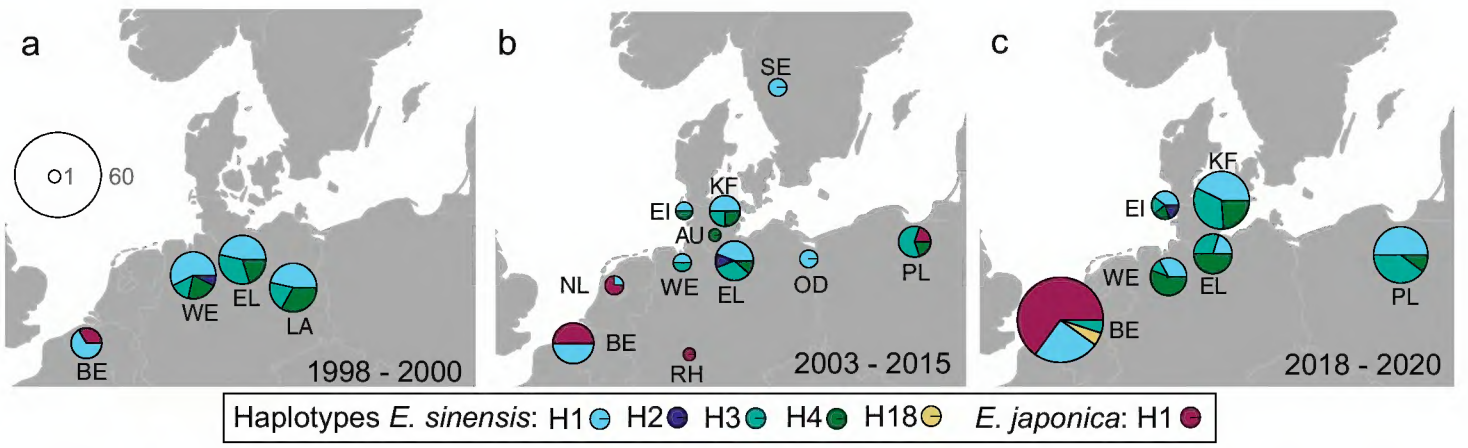

Figure 3. Geographic distribution of COI haplotypes of *E. sinensis* and *E. japonica* in the studied region of Europe, based on newly-generated and publicly-available sequence data **A** the WE (Weser River), EL (Elbe River) and LA (Laascher Lake) populations were sampled by Hänfling et al. (2002) and the BE (Belgium) population was collected in 1998 and used in this study **B** the NL (Netherlands), RH (Rhine River), PL (Poland), OD (Oder River) and SE (Sweden) samples were publicly available and previously synthesised by Hayer et al. (2019). The BE, EI, WE, EL, KF, AU and PL populations were sampled in this study and are detailed in Table 1 **C** all sequences were newly generated in this study and sample information is available in Table 1. Haplotype names correspond to Hayer et al. (2019).

df = 2, p-value = 0.28). Of the Polish samples, 20% (one of five specimens) carried the *E. japonica* haplotype in 2015 (Fig. 3B), but none carried this haplotype in 2020.

All the 41 investigated adult mitten crabs from Belgium, Germany and Poland with a carapace width from 44 to 96 mm were morphologically identified as *E. sinensis*: they had four distinct infraorbital teeth, the fourth lateral spine was pointing outwards, the epi- and protogastric crest was very strong and the carapace length to width ratio was between 1.019 and 1.158 (Suppl. material 1: Table S1, photographs available at 10.6084/m9.figshare.c.5341910).

The 35 examined juvenile crabs from Belgium and Germany were morphologically more variable (Fig. 4A and B). The ratio of carapace width to length was between 1 and 1.157 and, when tested with a linear regression model, did not differ significantly between specimens from Belgium or Germany (std. error = 0.02, t-value = -0.89, p-value = 0.29), specimens with either *E. japonica* or *E. sinensis* COI haplotype (std. error = 0.02, t-value = -0.23, p-value = 0.82) or specimens with different carapace width (std. error = 0.01, t-value = 1.38, p-value = 0.30).

The infraorbital teeth of the juvenile German *E. sinensis* crabs were sharply pointed in all but one individual (ID: KC09-90, Fig. 4C). In contrast, all but one juvenile crab from Belgium had more rounded infraorbital regions (Fig. 4C), without relationship to COI haplotype (std. error = 1.10, z-value = 0.29, p-value = 0.77) or carapace size (std. error = 0.11, z-value = 1.01, p-value = 0.31), based on the results of the logistic regression. Consequently, sampling locality (Germany vs. Belgium) significantly explained the shape of the infraorbital region (std. error = 1.33, z-value = 2.54, p-value = 0.01).

Similarly, the fourth lateral spines pointed outwards in all German individuals, but were less conspicuous in 55% (13 out of 28) of the Belgian individuals (Fig. 4D). The extent of the fourth spine was not related to locality (std. error = 0.01, z-value = –0.01,

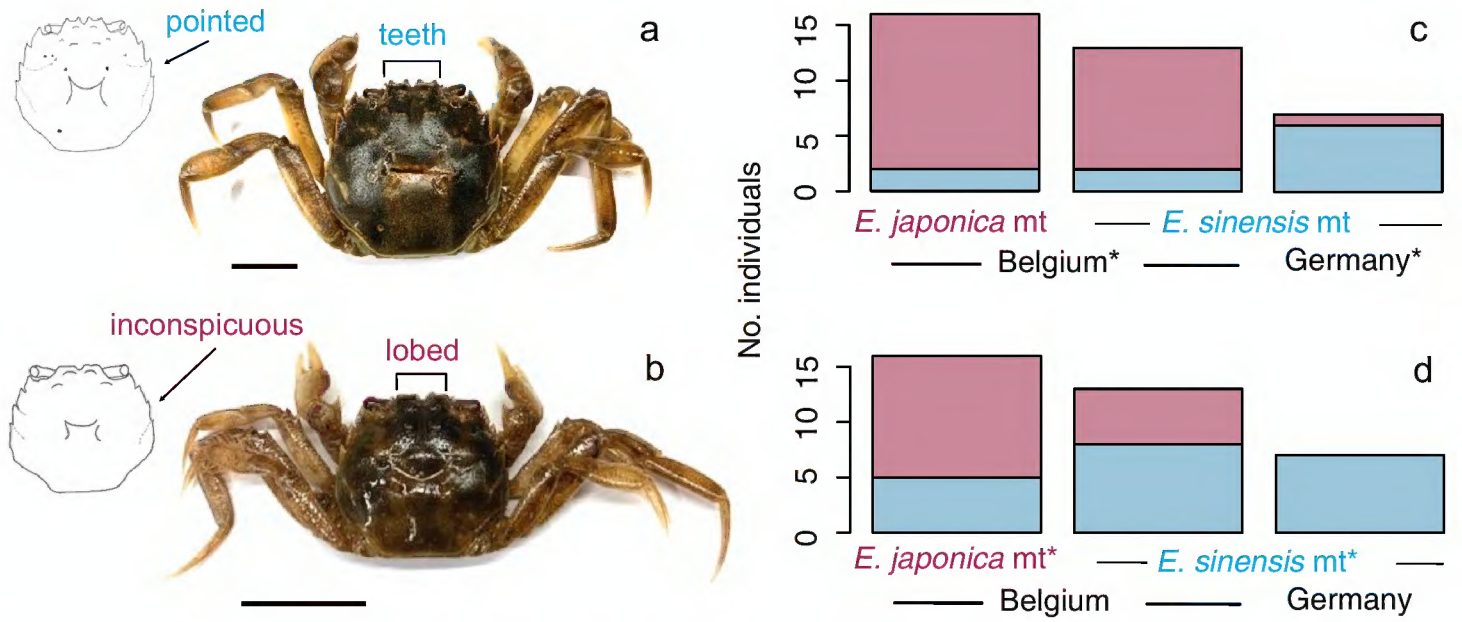

Figure 4. Morphological differentiation amongst juvenile mitten crabs from Germany and Belgium **A** juvenile crab from Germany (ID: KC20-02, collected in the Kiel Canal in 2020, *E. sinensis* mtDNA) with a toothed infraorbital region (brackets) and pointed fourth lateral tooth (arrow) **B** juvenile crab from Belgium (ID: BE20-05, collected in Belgium in 2020, *E. japonica* mtDNA) with a lobed infraorbital region and inconspicuous fourth lateral tooth. Scale bars indicate 1 cm. Distribution of morphological characteristics of infraorbital region **C** and fourth lateral tooth **D** in respect to mtDNA and origin of individuals. Significant differences between groups are marked by asterisks. Purple colour indicates Japanese mitten crab character states and mtDNA and blue colour indicates Chinese mitten crab character states and mtDNA.

p-value = 0.99) or carapace size (std. error = 0.09, z-value = –1.26, p-value = 0.21) in the logistic regression model, but to COI haplotype (*E. sinensis* vs. *E. japonica*) when locality was not included as an explanatory variable into the linear model (std. error = 0.82, z-value = –1.66, p-value = 0.01). The epi- and protogastric crest was strong, high and sharp in all German and most Belgian juveniles. Only four Belgian juveniles had a weakly-defined crest. The expression of this trait was not significantly different between individuals from different countries (std. error = 0.38, z-value = 0.01, p-value = 1.00) or COI haplotypes (std. error = 1.29, z-value = –0.79, p-value = 0.43) and did not vary significantly with carapace width (std. error = 0.13, z-value = –0.37, p-value = 0.71).

The geometric morphometric analysis was conducted on 69 specimens with a carapace width between 15 and 96 mm. Of these specimens, 29 were from Belgium, 27 from Germany and 10 from Poland. In addition, we included three specimens from the native range, two *E. sinensis* from the Yangtze River in China and one *E. japonica* from Shimonoseki in Japan (Fig. 1 shows two of these specimens). The first PCA axis explains 35% of the variability and correlates strongly with carapace width (Fig. 5). The ontogenetic morphological change occurring from juveniles to adults was already described by Peters and Panning (1933), especially with regard to the infraorbital region. In juveniles, the lateral and infraorbital teeth are less pronounced and the intraorbital distance is larger than in adults, which is apparent in the shape of the most extreme specimens in the PCA (Fig. 5A).

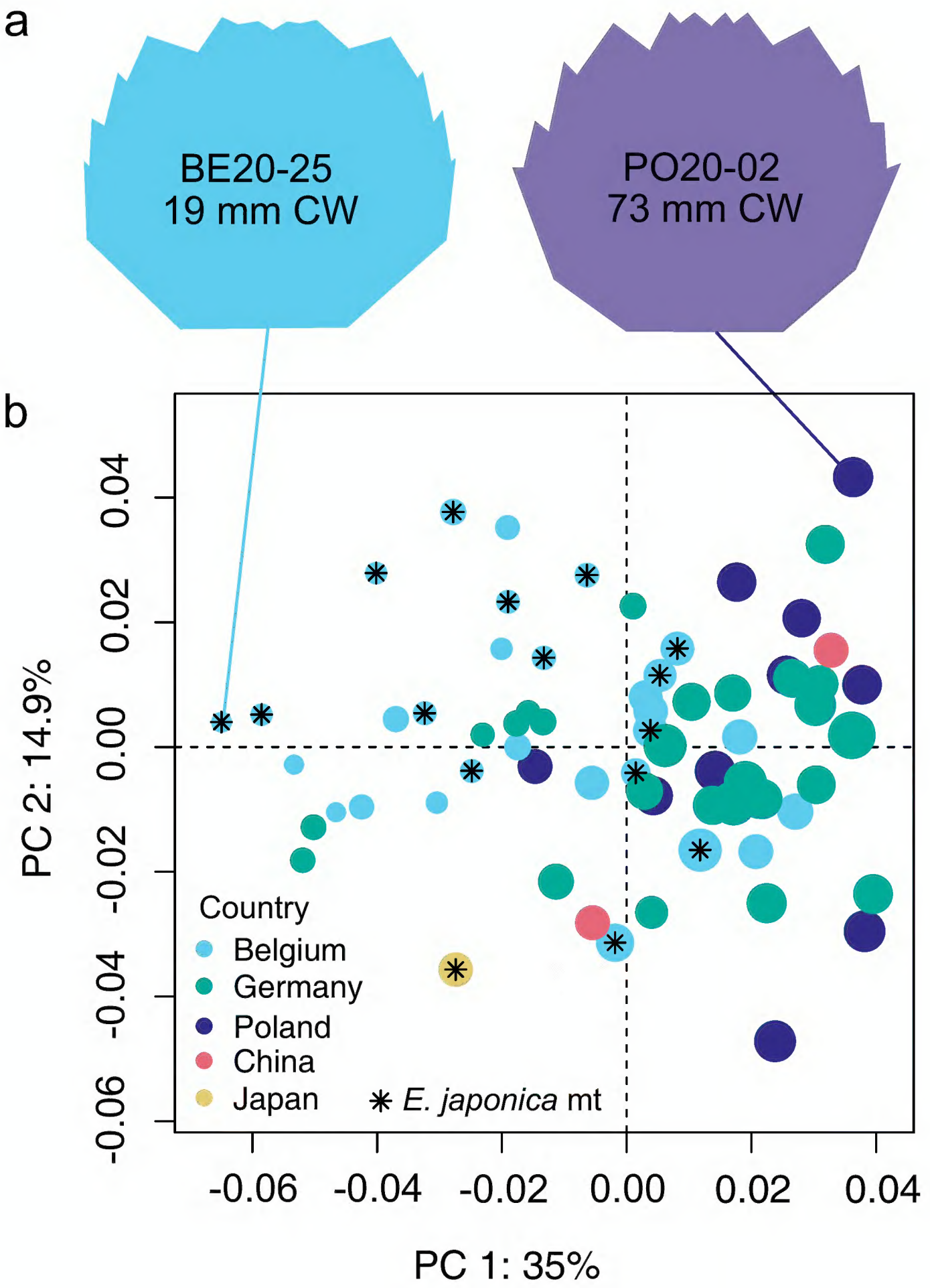

Figure 5. Geometric morphometric analysis on the carapace of mitten crabs from Belgium, Germany and Poland and three native specimens from China and Japan **A** procrustes shapes of two specimens at the opposite ends of the first principal component axis **B** scatterplot of the first two axes of the principal component analysis. Each dot is a specimen, with size proportional to carapace width. Colours denote different sampling countries and asterisks individuals with an *E. japonica* haplotype.

While the PCA does not indicate strong clustering by country, the analysis of variance gave significant results for both carapace width (p-value = 0.001) and locality (p-value = 0.001), but not for their interaction (p-value = 0.12) or mtDNA lineage (*E. sinensis* vs. *E. japonica*) (p-value = 0.16). These results remained the same after removing the native-range specimens from the analysis. The mitten crabs from Belgium, Germany and Poland clustered with the two Chinese mitten crabs from the native range. In the PCA, the Belgian juveniles (about half of which carried *E. japonica* mtDNA) were somewhat distinct from the German juveniles, for which the sample size was small (n = 7).

Discussion

Our study indicates the presence of Japanese mitten crab mtDNA in Belgium since at least 1998, possibly even earlier. Morphologically, however, all adult crabs are identified as Chinese mitten crabs, which explains why the presence of the genetic footprint of Japanese mitten crabs remained overlooked until the employment of molecular analysis. The juvenile crabs from Belgium showed morphological subtle differences to juveniles from Germany, but these differences do not match with the assignment, based on COI haplotypes. This mismatch between morphology and mtDNA implies introgression with a mostly *E. sinensis* nuclear genome, assuming that morphology reflects the nuclear genome well. The increase of *E. japonica* mtDNA from 33% to 65% from 1998 to 2020, observed in Belgian mitten crabs, indicates that this haplotype has become established in Europe and may become more abundant in the region. It likely became established also in the neighbouring Netherlands because the same Japanese mitten crab COI haplotype was found in three out of four mitten crabs collected there in 2011 (Hayer et al. 2019).

Many of the Belgian juveniles show morphological characteristics of *E. japonica*, while most juvenile mitten crabs from Germany show the characteristics of *E. sinensis*. As we did not find the *E. japonica* haplotype in any specimens from Germany or Poland, these populations may not have introgressed, suggesting that juveniles with more or less "pure" *E. sinensis* genomes may differ morphologically from juveniles with an introgressed genomic background. The PCA of the morphometric data also suggests that the morphological differentiation is more pronounced in juveniles. This proposed introgression could have occurred either in the native range, prior to their introduction, or after the introduction of "pure" parental Japanese mitten crabs to Belgium, where Chinese mitten crabs have been present since the 1930s. In support of the first hypothesis, a native hybrid zone exists at the northern limit of the two species' distribution around Vladivostok in Russia and northern China (Wang et al. 2008, 2013; Xu et al. 2009; Xu and Chu 2012). In this area, over 30% of mitten crabs are hybrids (Xu and Chu 2012).

The region around Vladivostok is a plausible source of the introduction of individuals carrying *E. japonica* mtDNA to Europe, whether pure or hybrid. We identified two individuals from Belgium with the *E. sinensis* haplotype H18 previously only detected in Vladivostok where 3 out of 10 specimens carried this haplotype (Xu et al.

2009; Hayer et al. 2019). The northern range of *E. sinensis* is only represented by Vladivostok in phylogeographic studies, so that we know little about the actual distribution of this haplotype. Nonetheless, Vladivostok itself, with its large port, is not an unlikely source population (Hayer et al. 2019). This could mean that multiple individuals were introduced from Vladivostok (or another locality where the H18 and the introduced *E. japonica* haplotype co-occur) at the same time, at least one with *E. sinensis* mtDNA (the H18 haplotype) and one with *E. japonica* mtDNA. Considering that ballast water was the likely vector, we assume that many larvae with each haplotype could have been introduced. Alternatively, multiple introduction events from different source populations might have occurred into Belgium, with at least one originating from an area where the H18 Chinese mitten crab haplotype occurs, for example, Vladivostok. Under this scenario, "pure" Japanese mitten crabs were introduced to Belgium at a time when Chinese mitten crabs were already present and both species hybridised subsequently in the introduced range. Given the high global shipping traffic to the ports of Belgium, both scenarios are likely. In fact, the scenarios are not mutually exclusive: both introgressed and pure parental species could have been introduced independently. This is alarming as it has resulted in new mitten crab genetic diversity in Europe with unknown effects on the invasiveness of the introgressed specimens.

Interestingly, each of the three European countries shows different temporal dynamics of COI haplotypes. In Belgium, the Japanese mitten crab COI haplotype seems to be increasing over time, though sample sizes are too small to confirm this increase statistically. In northern Germany, the same Chinese mitten crab haplotypes have been present in the past 23 years and the Japanese mitten crab haplotype has never been recorded. In Poland, on the other hand, the specimens sampled in 2020 had a different haplotype distribution to the specimens sampled in 2015. Mitten crabs do not form an established population in Poland where salinity is too low for larval development (Wójcik-Fudalewska and Normant-Saremba 2016). Instead, juveniles and adults may migrate across rivers each year from Germany to enter the eastern Baltic Sea (Czerniejewski et al. 2012). This hypothesis is supported by the haplotype distribution of the 2020 specimens from Poland, which was very similar to the German haplotype distribution. The mitten crabs from Poland sampled in 2015 had a very different haplotype distribution. These crabs may have been accidentally introduced by marine shipping from ports in Belgium and part of The Netherlands or directly from Vladivostok into Russian ports, like Baltiysk or Kaliningrad, located in the Vistula Lagoon. They are testimony to the highly dynamic invasion potential of mitten crabs, apparently due to their high adaptive plasticity (Cui et al. 2021) and explain why Chinese mitten crabs could spread across Europe within decades their initial discovery in Germany in 1912 (Schnakenbeck 1924).

Eriocheir japonica has a similar life history to *E. sinensis* with planktonic larvae and migrating adults, such that similar expansion rates may be assumed (Veldhuizen and Stanish 2002; Dittel and Epifanio 2009; Kalinina 2015). Given the rapid spread of *E. sinensis* within a few decades after its arrival in Europe, it is curious that the *E. japonica* mtDNA has not, to our knowledge, yet spread further than Belgium and The Netherlands. This could indicate that mitten crabs do not disperse between the Belgian and, for example, northern German river systems. Such limited connectivity was al-

ready proposed by Peters and Panning (1933), who stated that the Chinese mitten crab remained close to the mouth of a river, even during the planktonic larval phase. The rapid expansion of the Chinese mitten crab in the 1930s across many European countries seems to contradict this scenario, but may be explained by higher levels of anthropogenic dispersal caused by ballast water at that time, for which regulations only recently became stricter (IMO 2004).

Another possible interpretation is that the Belgian population with their introgressed Japanese mitten crab mtDNA has a lower invasive potential. Where the Japanese mitten crab itself has been reported outside its native range, for example, on the west coast of North America (Jensen and Armstrong 2004), the species did not become established. This could be due to a low ability for rapid adaptation, which is likely necessary for successful invasions (Lee 2002). On the other hand, "pure" Japanese mitten crabs are more aggressive than Chinese mitten crabs and win in direct competition for shelter (Zhang et al. 2019). Thus, where "pure" Japanese mitten crabs appear, they may become dominant. However, as the morphology indicates, the Belgian population does not consist of "pure" Japanese mitten crabs, but of Chinese mitten crabs with unknown levels of Japanese mitten crab genomic introgression. Furthermore, it is unclear whether or to what degree the Belgian introgressed population behaves differently from other non-native populations. Future studies should address both the extent of introgression as well as physiological and behavioural differences that could reflect differences in the invasive potential.

There is evidence that the invasion process of mitten crabs is ongoing (Rudnick et al. 2003). In addition to earlier introductions to Europe and the West coast of the United States, *E. sinensis* has colonised Portugal, Spain, Italy and Ireland in the last decades (Cabral and Costa 1999; Rudnick et al. 2003; Garcia-de-Lomas et al. 2010; Crocetta et al. 2020) and has been reported in the Tokyo Bay of Japan (Takeda and Koizumi 2005). *E. japonica* has been reported on the west coast of North America (Jensen and Armstrong 2004) and *E. hepuensis*, native to China, has been introduced to Iraq (Naser et al. 2012) and Iran (Naderloo 2014). These new findings warn us that mitten crabs are still on the move. The role of introgression for the invasive potential in this species has not been considered. Given the fact that *E. sinensis* is a recognised global invasive species (e.g. it has been included in the list of invasive alien species of European Union concern; European Commission 2016), understanding the ecological consequences of this introgression and its geographic extent might contribute for the development of an effective control and management strategy.

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Supplementary material 1

Table S1. Master spreadsheet with morphological and genetic information for each specimen

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Data type: Morphological.

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